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Effect of Environmental Factors on Monocarbonyl Potential in Fresh Bovine Milk Fat [1]

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Abstract

A quantitative study of various environmental and physiological factors on the monocarbonyl potential in heat-treated bovine milk fat was conducted. Analyses of monocarbonyls (as their 2,4-dinitrophenylhydrazones) indicate a definite seasonal trend, being higher in the winter than in the summer. A highly significant ($P < 1\%$) positive correlation between the monocarbonyls and the aliphatic delta-lactones occurred over season.

Analyses of weekly samples throughout a complete lactation of the monocarbonyls and methyl ketones indicated a positive correlation ($P < 1\%$) with the aliphatic delta-lactones and short-chain fatty acid (4:0-14:1) production. No significant difference in total monocarbonyl potential from fats of different breeds, fat production, and milk from ketotic animals could be shown. These data aid in characterizing the variability in formation of the nonoxidative compounds in milk fat and further lend support to the evidence that their precursors arise from, and are controlled by the availability of acetate during the biosynthesis of fatty acids.

Aliphatic monocarbonyl compounds; namely, the methyl ketones, alkanals, 2-enals, and 2,4-dienals in bovine milk, play an important role in the ultimate flavor of stored and heated dairy products (3, 4, 9) and undoubtedly contribute to the flavor of fresh pasteurized beverage milk (5). However, no specific name has been given to the flavor they impart, unless it is a loose inclusion under the term stale. The characterization of the monocarbonyls in the lipid phase of milk is complexed by the fact that they may originate from numerous sources. The alkanals, 2-enals, and 2,4-dienals have been identified in autoxidized dairy products and are thought to arise as a result of dismutation of hydroperoxides formed in the fat from unsaturated fatty acids. The mechanisms for the formation of methyl ketones in milk fat are not completely understood. There is, however, sufficient evidence to show that the methyl ketones of odd-numbered carbons from C_3 to C_{15} are a result of a nonoxidative mechanism via an endogenous pathway (3, 4, 9) and are not due to an autoxidation breakdown as first theorized (9).

The concentration levels of the odd-numbered carbon methyl ketones (for bovine milk fat) reported in the literature (4-6, 10) indicate variation and it is suggested that this may be due to a seasonal effect (3, 7).

This study was undertaken to obtain a better understanding of the variation in the monocarbonyl fraction of bovine milk fat and its relationship to the aliphatic delta-lactones with respect to certain environmental and physiological conditions.

Experimental Procedure

Preparation of butteroil. All milk samples used in these experiments were obtained from the University Herd. Following separation, fresh cream (37-40% fat) was churned in a Waring Blendor at 4 C. The butter granules were warmed and centrifuged at 2,000 rpm (International Centrifuge, Model PR-1) for 30 minutes to obtain serum-free butteroil. Immediately following centrifugation a known amount of oil was weighed (approximately 5 g) into screw-capped, poly-seal glass vials. These vials were then heat-treated at 100 C for 6 hours. This heat treatment with sufficient water retained in the oil promoted hydrolysis of the β-keto acid precursors from the glyceride and was necessary for total methyl ketone formation (14).

Analysis of monocarbonyl fraction. The heated butteroil samples were dissolved in 200 ml of purified hexane. Fisher Certified hexane was purified by refluxing with sulfuric acid; distilling in glass over KOH pellets, and rendering carbonyl-free according to the method of Schwartz and Parks (12). The hexane extract was then passed over a Celite column impregnated with DNP-hydrazine, H_3PO_4, and H_2O as described by Schwartz et al. (11) to convert

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the carbonyls to DNP-hydrazones. The DNP-hydrazones were completely eluted from the column with 200 ml of purified hexane. The lipids were removed from the DNP-hydrazones by passing the mixture over a Celite 545-Sea Sorb 43 (Fisher Scientific Company, Pittsburgh) column (1:1) (11), using a total of 20 g of column packing material. Two hundred milliliters of hexane rendered the DNP-hydrazones free of lipids. The adsorbed monocarbonyl derivatives were eluted from the column with 140 ml of 3:1 chloroform-nitromethane. Ketoglyceride derivatives were separated from the monocarbonyl DNP-hydrazones on an alumina column (12). Forty grains of neutral alumina (80-100 mesh) activated by heating 24 hours at 150 C, partially deactivated by adding 6% (w/w) distilled H_2O, and allowed to equilibrate 16-20 hours, was necessary for good separation.

The resulting monocarbonyl DNP-hydrazones were evaporated to dryness and dissolved in a known volume of chloroform. The concentration was determined by measuring absorbency of the solution against chloroform at 365 mμ and converting the reading to micromoles using E = 22,500 (8). All readings were done with a Beckman Recording Spectrophotometer, Model DB-G.

Analysis of methyl ketones The methyl ketone DNP-hydrazone class was isolated from the above monocarbonyl fraction by the procedure of Schwartz et al. (13). A 20-g column was prepared using Magnesia 2665 (Fisher Scientific Company) and Celite 545 (1:1); and separating methyl ketone derivatives from trace amounts of aldehydes with the following solvents: 150 ml of 15% chloroform in hexane, 150 ml of 30% chloroform in hexane, 100 ml of 60% chloroform in hexane, and 150 ml of 100% chloroform. A fraction collector equipped with a UV source to monitor the eluant from the column aided in determining the separation. The methyl ketone DNP-hydrazones were evaporated to dryness, pooled, dissolved in known volume of chloroform, and read spectrophotometrically to determine the concentration.

Results and Discussion

This series of experiments was conducted in conjunction with another study in which the aliphatic delta-lactones were analyzed against the same parameters and utilized the same sources of fat (1). For a better definition of the nonoxidative changes which occur in bovine milk fat, that is the formation of both methyl ketones and aliphatic lactones, the results will be discussed in light of data concerning the lactones.

Seasonal variation. Figure 1 (upper) graphically illustrates the monocarbonyl concentration throughout a 52-week period from milk sampled weekly. The data for the variability in monocarbonyl concentration indicate a definite similarity during the test year when compared to the lactone potential (Fig. 1, lower). A cubic regression equation best described the data for both classes of compounds and resulted in a highly significant positive correlation ($r = 0.608^{**}$) between the monocarbonyls and lactones over a season. Hence, the monocarbonyl concentration (μmoles/10 g of fat) follows the same seasonal trend, being higher during the winter months (barn-feeding) and lower during the summer months (pasture-feeding). These data lend indirect support for mechanism for formation of methyl ketones in milk fat by Lawrence and Hawke (7); that is, the C_5-C_{15} methyl ketone precursors are synthesized from acetate during biosynthesis of fatty acids. This is based on the fact that the seasonal trend of the monocarbonyl fraction, increasing from a low level during May, June, and July, to a high concentration during November and December and then decreasing, follows the same seasonal trends in mole per cent distribution of the short-chain fatty acids in

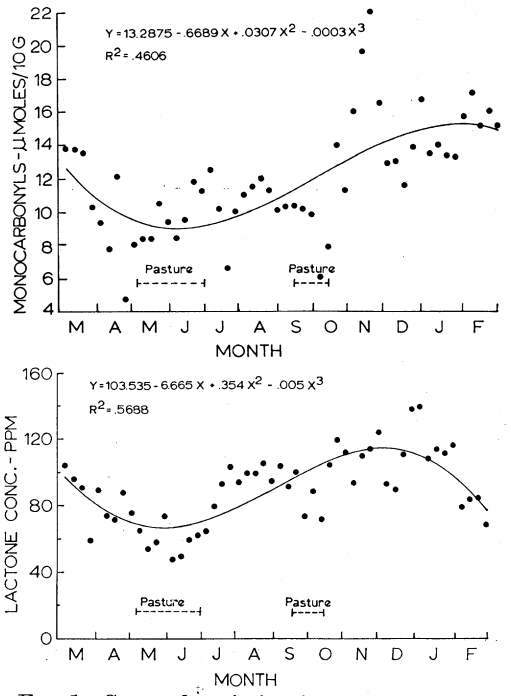

FIG. 1. Seasonal variation in monocarbonyl potential (upper) and aliphatic delta-lactone potential (1) (lower) of bovine milk fat.

milk fat as shown by Hansen and Shorland (2). As speculated by Lawrence and Hawke, the amounts of methyl ketone exhibit a seasonal variation, since both C_5-C_{15} methyl ketone precursors and the short-chain fatty acids of milk fat are synthesized from acetate. Unknown environmental and physiological factors, other than feed and stage of lactation (1), such as ambient temperature, relative humidity, and light may play a role in producing the sine-like curves for the seasonal data. Over the entire sampling period, the monocarbonyls averaged 12.0 ± 3.4 μmoles/10 g of fat.

Stage of lactation. Butteroil samples were isolated from morning milkings collected weekly throughout a 310-day lactation from one Holstein cow. Figures 2 and 3 present the data for the concentrations of the monocarbonyls and methyl ketones throughout lactation. Linear regression equations best described the data and also indicated a high significant difference ($P < 1\%$) between stage of lactation and the monocarbonyl and methyl ketone production. Throughout the complete lactation the monocarbonyls and methyl ketones in the milk fat averaged 13.2 ± 3.3 μmoles and 10.0 ± 3.6 μmoles/10 g of fat, respectively, indicating methyl ketones were the major class (76%) in the monocarbonyl fraction. Analyses of more than 100 samples during the entire study indicated the monocarbonyls were composed of from 75-90% methyl ketones with trace amounts of aldehyde present. The absorption maxima of all the monocarbonyl fractions isolated peaked at 365 mμ against chloroform, suggesting a high proportion of methyl ketones in relation to the monocarbonyl classes derived via autoxidation.

The correlation coefficients between the nonoxidative compounds formed in the milk fat and some of the variables tested during lactation are presented in Table 1. These data reveal two important points; first, there is significant correlation between the amounts of monocarbonyls, methyl ketones, and aliphatic delta-lactones produced in milk fat, which lends confirmation to the results obtained in the seasonal study; and secondly, there is similarity in the degree of significance and direction (positive or negative) of the correlation coefficients between the amounts of nonoxidative compounds and the variables tested during lactation. Here again, the role of acetate in the formation of the methyl ketone precursors is evident, as seen in the highly significant positive correlation with short-chain fatty acid production throughout lactation.

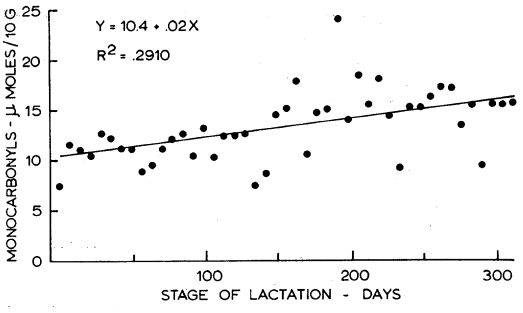

FIG. 2. Variation in monocarbonyl potential of bovine milk fat with stage of lactation.

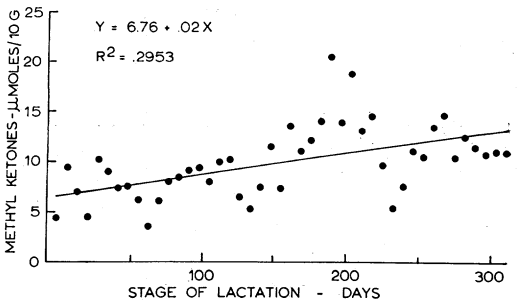

FIG. 3. Variation in aliphatic methyl ketone potential of bovine milk fat with stage of lactation.

Variation among breeds. Table 2 contains data on the monocarbonyl concentration for three breed groups tested. There was a high variability within each group, although no significant difference could be shown between the three breed groups, even when the time of sampling was removed. Likewise, no significant difference was evident for the mean lactone potentials (1) of the same fat for these groups. However, concentrations of the monocarbonyls

TABLE 1

Correlation coefficients between lactone, monocarbonyl, and methyl ketone potentials and tested variables

Variables	Aliphatic delta-lactones	Monocarbonyls	Methyl ketones
	(r)		
Stage of lactation	0.636**	0.555**	0.543**
Fat test	−0.611**	−0.386**	−0.401**
Milk yield	−0.021	−0.226	−0.218
Fat yield	−0.443**	−0.406**	−0.435**
Short-chain fatty acids	0.829**	0.388**	0.443**
Aliphatic delta-lactones	.....	0.308*	0.441**

* 5% level of significance $r = 0.291$.
** 1% level of significance $r = 0.376$.

TABLE 2

Monocarbonyl concentration of butteroil from various breeds of dairy cattle[a]

Sample period	Holstein (40)[b]	Brown Swiss (34)	Jersey-Guernsey (27-25)
	——concentration-μ moles/10 g——		
1	14.0	15.2	11.9
2	7.3	9.2	9.3
3	14.8	11.4	16.2
4	[c]	17.6	16.0
5	18.3	15.3	15.7
6	15.2	15.2	18.2
7	17.2	6.3	15.6
8	9.9	14.5	13.6
9	16.4	16.0	16.3
Mean±S.D.	14.1±3.8	13.4±3.7	14.8±2.7

[a] Samples collected from breeds on identical feeding regimens.
[b] Number of animals representing breed.
[c] Laboratory accident.

were lower in the Brown Swiss fat, as were the aliphatic delta-lactones.

Variation due to fat production. Results (Table 3) relating to the influence of fat production on monocarbonyl concentration indicated no significant difference between the two groups.

Variation due to ketosis. Monocarbonyl concentrations of milk fats collected from cows when in a normal and ketotic condition indicate similar levels (Table 4). However, as would be expected, the level of acetone in the milk from ketotic animals would be high, due to the formation and accumulation of ketone bodies in the gland and, therefore, quantitatively offset the low levels of the C_5-C_{15} methyl ketones. Preliminary evidence, shown by gas and thin-layer chromatography, indicates a high proportion of acetone (75-90%) in relation to the C_5-C_{15} methyl ketones in milk fat from ketotic cows. Similarly low levels of carbonyls were found in the steam distillate of ketotic milk fat (1).

TABLE 3

Monocarbonyl concentration of butteroil from high vs. low fat producing cows[a]

Sample period	High fat producers[b]		Low fat producers[c]	
	Cow 1	Cow 2	Cow 3	Cow 4
	——concentration-μ moles/10 g——			
1	12.2	15.5	13.6	13.4
2	14.0	14.7	13.7	14.7
3	16.6	22.4	19.7	18.6
4	20.0	16.3	19.5	20.1
5	18.4	19.8	12.9	17.8
6	19.7	16.1	17.9	17.6
Mean±S.D.	17.1±3.0		16.6±2.8	

[a] Grouped according to previous lactation records. All animals within 26 days of lactation of each other.
[b] Fat yield based on milk taken during sampling —697 ± 133 g.
[c] Fat yield based on milk taken during sampling —484 ± 167 g.

TABLE 4

Monocarbonyl concentration of butteroil from cows when in a ketotic vs. normal condition

Cow	Ketotic	Normal
	—concentration-μ moles/10 g—	
1	16.1	12.2
2	14.5	16.2
3	18.3	16.5
4	12.7	11.0
Mean ± S.D.	15.4±2.4	14.0±2.8

Conclusions

These findings indicate that the potential of milk fat to produce monocarbonyls, namely the odd-numbered carbon aliphatic methyl ketones, as a result of moist heat, is influenced by environmental conditions, such as season and stage of lactation. The highly significant correlations between the monocarbonyls and delta-aliphatic lactones analyzed under various parameters lend supporting evidence that their precursors arise from, and are controlled by the availability of acetate during the biosynthesis of fatty acids. The gross similarities between the two nonoxidative classes of compounds suggest a dual contribution in the susceptibility of a dairy product to develop a stale flavor. These findings enumerate the importance of elucidating the biochemical origin of flavors and their precursors, and determining those factors which control their formation. This knowledge is necessary to better regulate the flavor potential in food products.

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